412.34

FILE COPY
FOREST INSECT LABORATORY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA

UNITED STATES DEPARTMENT OF AGRICULTURE

BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE

FOREST INSECT INVESTIGATIONS

CRITICAL LOW TEMPERATURES FOR THE BLACK HILLS BEETLE

1936-37

By

N. D. Wygant, Assistant Entomologist

Forest Insect Laboratory,
Fort Collins, Colorado,
November 26, 1937.

JMM
KAS
JEP
GRS
PCJ
ASW

U. S. GOVERNMENT PRINTING OFFICE 00918

CRITICAL LOW TEMPERATURES FOR THE BLACK HILLS BEETLE

1936-37

By

N. D. Wygant, Assistant Entomologist

Forest Insect Laboratory,
Fort Collins, Colorado,
November 26, 1937.

CONTENTS

	Page No.
Introduction	1
Equipment	2
Methods	3
Results of Experiments	
Experiments to Determine Survival After Exposure to Cold	8
Critical Temperatures in Relation to Season	11
Critical Temperatures in Relation to Host	13
Critical Temperatures in Relation to Stage of Development	15
Summary of Weather Records	16
Rearing of Black Hills Beetle in Greenhouse	17
Summary and Conclusions	18
Appendix	21

CRITICAL LOW TEMPERATURES FOR THE BLACK HILLS BEETLE

1936-37

INTRODUCTION

Low winter temperatures have long been recognized as an important factor limiting the distribution of certain species of insects in the temperate climates. Similarly abnormal or unseasonal low temperatures are frequently fatal to a large portion of the overwintering broods of some insects. In some cases these cold periods have been one of nature's valuable checks to barkbeetle increase and have brought outbreaks under control or checked the impetus of the epidemic. The low temperature studies were undertaken to determine the effect of low winter temperatures on the Black Hills beetle (Dendroctonus ponderosae Hopk.) population and its relation to epidemics and subsequent timber losses. It is important to know in the case of this serious forest insect pest how well it can resist winter temperatures so that the knowledge can be used in planning artificial control measures. In the case of some barkbeetles the mortality has been high enough to make it possible to abandon control operations during certain years.

The low temperature studies can be conducted more satisfactorily in the laboratory where temperatures can be controlled accurately by special refrigeration apparatus than in the field where there is no

control over the temperatures and where the occurrence of low temperature periods are unpredictable. The information gained through the laboratory study can be applied to natural field conditions and used to predict what will happen to the brood under the bark during periods of abnormal or unseasonal temperatures.

Considerable work has been done on the study of cold-hardiness of some species of Dendroctonus, particularly in California and Oregon, but the studies reported in this paper are the first on this subject dealing with the Black Hills beetle (D. ponderosae Hopk.).

The low temperature experiments for the season of 1936-37 were outlined to determine the critical temperatures of the Black Hills beetle at various times of the season, and to determine the critical temperature range of the insect in relation to its hosts of the region.

EQUIPMENT

The experiments were carried out in an automatically controlled, well type, low temperature cabinet designed by the Forest Insect Laboratory at Berkeley, California. The controlled range of the cabinet is from 25° F. to -25° F., however lower temperatures can be reached by "hand-controlling" the compressor. The calibration on the control dial was not accurate at the lower temperatures, especially between 0° and -25°. The temperature inside the cabinet was determined by a thermometer and the temperature control set by the thermometer readings rather than

by the calibration on the dial. A Weather Bureau type minimum thermometer was inserted through a hole in the wall of the machine provided for thermocouples so that the entire thermometer was inside the cabinet. Temperature readings could thus be taken without opening the lid of the machine. The thermometer accurately recorded the temperature inside the cabinet in about 10 minutes, therefore reliable readings were taken every 15 minutes. A second minimum thermometer was placed inside the cabinet with the insects at the beginning of each exposure period and taken out and read at the end of each period. This thermometer recorded the lowest temperature to which the insects were exposed. While the thermometers do not measure the temperatures as precisely as desired, they provided the best means available for regulating the cabinet at the low temperatures. A potentiometer with thermocouples has been purchased to use in the experiments to be continued the next season and should increase the precision in controlling the temperatures.

METHODS

The methods used for the most part in making the exposures of the larvae to the low temperatures were those illustrated by Mr. Yuill of the Forest Insect Laboratory at Berkeley. The technique was adapted and improved upon during the winter, as the experiments progressed, to fit the conditions for best results with the Black Hills beetle. Mr. Yuill spent several weeks in Fort Collins during the latter part of

October getting the apparatus in working order and giving valuable instructions on the technique. One complete test was made while he was here.

Considerable trouble was experienced with the apparatus and technique and was reported upon in a memorandum 1/, therefore discussion of this matter will not be included in this report.

Most of the insects used in the tests were obtained from infested pine stored in an old shed at Allenspark, Colorado. The shed was well ventilated by large cracks in the sides and an open front and merely protected the logs from snow, rain, and woodpeckers. The elevation of Allenspark is approximately 8,500 ft. and is about the same elevation as the localities from which the infested pine was collected. The material was not stored at Fort Collins (elevation 5,000 ft.) because the climate is different from the higher elevations where the pine forests naturally grow. Allenspark is about 60 miles by highway from the laboratory.

The infested ponderosa pine was obtained from an infestation on Deer Ridge near Allenspark on November 2 and 3, and stored in the shed. In several tests the grubs from ponderosa pine were collected in the field near Allenspark rather than from the stored material. The Forest Service carried on control operations all winter and it was much easier

---

1/ Discussion of Critical Low Temperature Tests of Black Hills Beetle, December 1936. Memorandum for the files. N. D. Wygant.

to get the grubs directly in the field when weather permitted.

The limber pine was obtained from Boulder Ridge on November 4, taken to Allenspark and stored on November 14. The elevation of Boulder Ridge is 8,500 ft. and it is in the northern part of the Roosevelt National Forest, approximately 50 miles north of Allenspark. The supply of limber pine was exhausted before the spring tests were made and two tests were made with insects collected in the field near Allenspark.

The infested lodgepole pine was from Mullison Creek (elevation approximately 8,800 ft.) on the Medicine Bow National Forest, Wyo., and was collected November 18. The material was packed out in slabs from 12" to 18" DBH trees and brought to Fort Collins where the slabs were covered with hot paraffin wax to prevent drying out. The wax-covered slabs were then taken to Allenspark and stored with the other material.

The material from the three hosts was stored at the same place so that all would be subject to the same weather conditions, and also to facilitate the collection of the larvae for the tests. Very rarely does an infestation occur in the three hosts in one locality.

An effort was made to select larvae that were at least half-grown and preferably three-quarters to full grown, because larvae of such size, according to Mr. Yuill who worked with the mountain pine beetle, are stronger and better able to withstand the unnatural conditions they are subjected to during the tests. The ponderosa pine larvae used in the tests were nearly all three-quarters to full grown and many had started to construct their pupal cells before winter dormancy.

Ponderosa pine trees with such large larvae were not in the majority, and fortunately for the experiments, there were a large number of infested trees from which to choose those with the larger grubs. The accessible infested limber pine was limited and the largest larvae from this host that could be found for the tests were hardly half-grown and most of the infested trees from which they were chosen contained very small larvae. The lodgepole pine larvae were slightly smaller than the ponderosa pine larvae although many of them had started their pupal cells.

The larvae that were taken from the bark in the field were placed in ointment tins with 50 paraffin cells, one larva in each cell (see Fig. 1, page 19). The ointment tins were placed in a larger tin container packed in snow and transported to Fort Collins and stored in a refrigerator at 35° to 40° F. until used. During the winter when it was too cold to work in the field, a sufficient number of the infested logs stored at Allenspark was brought to Fort Collins and the larvae taken from the bark in a laboratory at approximately 70° F. The logs were stored out of doors and were in the laboratory only while the larvae were being removed. As soon as a dish was filled with the 50 larvae it was stored in a refrigerator, or in an unheated garage when the weather was not too cold, until used. The insects were kept at developmental temperatures no longer than absolutely necessary.

The insects were exposed for 2 hours and 15 minutes as naked larvae in lots of 50 in paraffin cells, one in each cell, in petri dishes (see Fig. 2, page 19). Mr. Yuill in his work observed that it required

approximately 15 minutes for the temperature in the petri dishes to reach that in the cabinet. The larvae were actually exposed for 2 hours at the cabinet temperature. The cabinet was held at a constant temperature during the exposure and exposures were made at each $2\frac{1}{2}^{0}$ F. interval over the critical range. Most of the tests were started at the ultimate critical point, when it was possible for the cabinet to reach it, and then each succeeding test run at each $2\frac{1}{2}^{0}$ interval higher. When working at the low temperatures far less time was required for the cabinet to warm up $2\frac{1}{2}^{0}$ than to go down $2\frac{1}{2}^{0}$. In fact at the lowest temperatures no time was lost between the tests because the taking out of the exposed larvae and putting in a new batch of unexposed larvae would raise the temperature in the cabinet $2\frac{1}{2}^{0}$.

In the first tests a count was made of the frozen larvae immediately upon being taken from the cabinet. The frozen larvae were frosty white and frozen solid, while the unfrozen ones were cream colored. During mid-winter, however, the larvae are more dormant and the frozen larvae are not easily distinguished from the unfrozen ones. Therefore the "immediate" counts were not taken on all of the tests. Then, too, it was necessary to take the lid off the petri dishes to make the counts and the sudden exposure of the insects to room temperature after being taken from the cabinet may be a severe shock. In the first tests the larvae were placed at room temperature as soon as taken from the cabinet, but in later tests they were put into a petri dish container, packed in ice, and allowed to warm up to 32^{0} F. over a period

of 2 hours before being placed at room temperature. Mortality counts were taken at 24 hour intervals after removal of the insects from the cabinet and the count showing the greatest number of living insects was used in interpreting the results.

RESULTS OF EXPERIMENTS

The tables containing data on the results are included in the appendix for convenience and to simplify the main text.

Experiments to Determine Survival after Exposure to Cold

The greatest difficulty encountered after exposure was in determining when the larvae were dead. With the larvae used by Mr. Yuill in the tests made at Fort Collins on October 28 and 29, 1936 it was relatively easy to differentiate between the living and dead grubs. The grubs wiggled vigorously when observed at room temperatures. The larvae had become much more dormant by December and many showed no signs of movement even when left at 70° F. for several hours. Some showed very weak motion; some a trembling action detectable only under a binocular microscope; while others showed rather vigorous activity.

In the tests made on February 4 and 5, 1937 duplicate samples of ponderosa pine larvae were exposed at the various temperatures and half of the samples placed in a refrigerator for 4 weeks after exposure before mortality counts were made. It was thought that by leaving the

larvae in the refrigerator for approximately 4 weeks the ones killed by the cold would be decomposed sufficiently to distinguish them from the living unfrozen ones, and that the supposedly dormant ones would show more activity. The results of this test are presented in the following table.

Temperature Deg. F.	Mortality Percent Larvae Placed at Room Temperature Immediately	Mortality Percent Larvae Stored in Refrigerator 4 Weeks
-17½	10	6
-20	24	20
-22½	46	18
-25	58	24
-27½	48	38
-30	70	28
-32½	68	34
-35	66	48
Check	4.7	5

There was a lower mortality count among the larvae stored in the refrigerator for 4 weeks after exposure than among the larvae placed at room temperature immediately after exposure. The procedure did not obtain the desired results as the same difficulty existed in separating the living larvae from the dead ones.

Although many of the larvae froze solid when exposed to the lowest temperatures, many showed signs of life when warmed up. To determine whether these frozen larvae would be able to continue development, test No. 6 (see appendix) was made on February 18 and 19, 1937.

Duplicate samples, 50 each of ponderosa pine larvae, were exposed in petri dishes over the critical range at 5° F. intervals. Half were placed at room temperature after exposure for mortality counts as had been done in the previous experiments. The other half were placed in 9x36 mm. vials (see Fig. 3, page 20) with fresh phloem within several hours after the exposure period. The vials were closed with cotton and placed in 8-ounce ointment tins. Some of the larvae soon started feeding on the phloem and developed into adults while others were unable to feed even though they were alive. Those that were alive and unable to feed gradually wasted away, some lived as long as a month. Apparently the low temperatures injured some of the larvae beyond recovery. The results of this preliminary experiment are presented in the following table.

Temp. Deg. F.	Larvae in Petri Dishes: No. Larvae in Sample	Larvae in Petri Dishes: Mortality Percent	Larvae in Vials with Phloem: No. Larvae in Sample	Larvae in Vials with Phloem: Mortality Percent
-15	50	4	50	8
-20	50	4	50	18
-25	50	16	50	66
-30	50	40	50	100
-35	50	30	50	96
Check	100	5	50	0

It will be noted that the mortality percents of the larvae in the vials with phloem are much higher than in the petri dishes and that all of the 50 check larvae developed to adults. This indicates that some of the larvae are not killed directly by the cold but are disabled and cannot

continue development. Some of the disabled ones showed vigorous movement but they were unable to feed and develop. Since all of the check larvae were able to continue development, the disabled ones were considered killed by the cold. Apparently the larvae injured by the freezing were the ones confusing the mortality counts in the petri dishes in the earlier tests.

The results of this preliminary feeding test were so encouraging that the next two succeeding tests were also made with the vials and phloem to check with the old method of making activity counts in the petri dishes. Test No. 7 (see appendix for date) gave equally good results with the feeding tests. Test No. 9 which was made on April 27-30 after the insects had become active with the advance of the season gave similar mortality percents with the two methods, indicating that the feeding tests will be necessary only during the winter when the insects are much more cold-hardy and dormant.

Critical Temperatures in Relation to Season

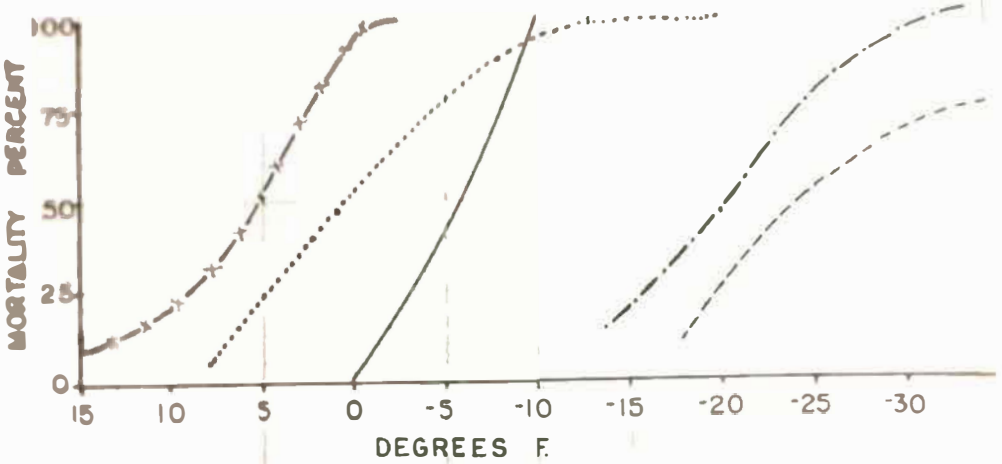
Chart I presents graphically the seasonal hardiness in the three hosts. The December tests (Test No. 3 in the appendix) were not included because of the erratic results. Tests made on February 4 and 5 (Test No. 5 in appendix) are included but the data are probably not very reliable because they were taken from mortality records determined by activity counts in the petri dishes before the feeding test method with the vials and phloem was used.

The fall critical temperatures were determined for the larvae from ponderosa pine only. The critical range on October 28 and 29 was from 0° to -10°, but by February the larvae were much more cold-hardy, as indicated on the chart. By March 17 the larvae had apparently lost some of their cold-hardiness, particularly the larvae from limber and lodgepole pine. By the last of April the insects were less hardy than during March and by the middle of June they were even less hardy than they were in April. The larvae become more cold-hardy as winter approaches and then gradually lose the hardiness as spring and summer approach. The fall and early winter tests are not complete enough to get a complete picture of gradual cold tempering. During midwinter the larvae are very dormant and even after several hours of exposure at room temperature may show no signs of life. During extreme dormancy the larvae are straight and do not curl up in a horseshoe-shaped position, characteristic of Scolytid grubs.

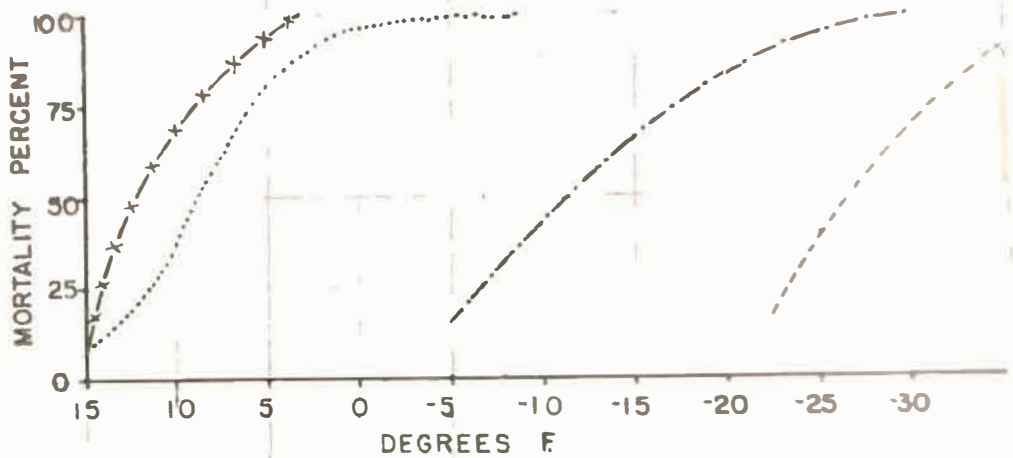
CHART I

CRITICAL TEMPERATURES IN RELATION TO SEASON

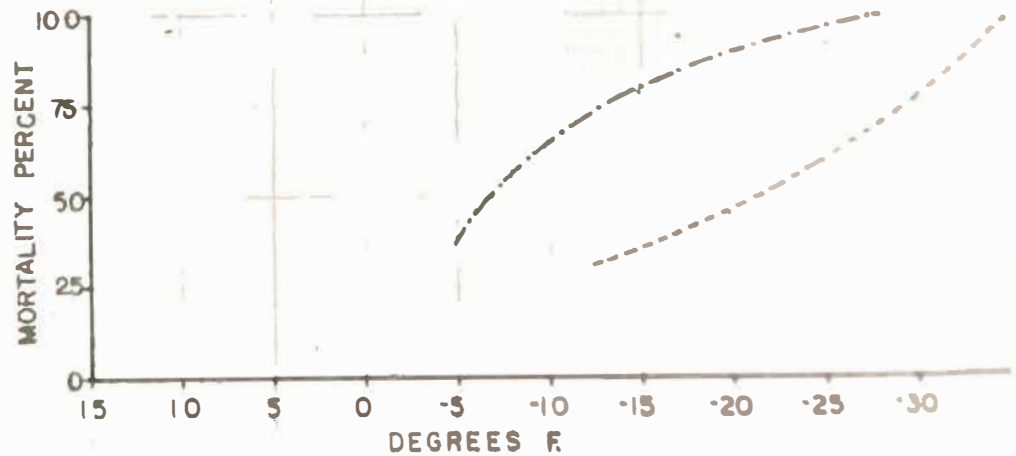
HOST PONDEROSA PINE



HOST LIMBER PINE



HOST LODGEPOLE PINE



LEGEND

OCT. 28, 1936
FEB. 4, 1937
MARCH 17, 1937
APRIL 27, 1937
JUNE 15, 1937

Critical Temperatures in Relation to Host

Chart II presents graphically the relation of cold-hardiness to the host in which the brood developed. Although the hosts are from different localities they are from approximately the same elevation. They were stored at one locality and were subject to the same winter weather conditions. They were collected the first part of November and it may be possible that the local climates had different hardening effects prior to the collection. The supply of lodgepole pine was small and not sufficient for tests throughout the winter.

As stated before, the tests on February 4-5 may not be reliable because they were made before the feeding test method was used. The tests at that time show the ponderosa pine larvae to be somewhat more hardy than those from the other two hosts.

The March 17-18 tests should be more reliable as the results are based on feeding tests, or ability of the larvae to feed and develop after being exposed to the low temperatures. As shown on the chart the ponderosa pine larvae were at that time approximately 12° more cold-hardy than the lodgepole pine larvae and approximately 9° more hardy than the limber pine larvae.

The April 27-30 tests show about the same difference in cold-hardiness between the ponderosa pine and limber pine larvae as the March tests. Unfortunately larvae from lodgepole pine tempered under the same weather conditions were not available.

The tests on June 15-17, when the insects were in a very active

stage, showed a difference of 5^{0} to $7\frac{1}{2}^{0}$ hardiness over the critical range between ponderosa and limber pine larvae, the larvae from the former host being the more hardy.

Apparently the food from the three hosts has an important bearing on cold-hardiness. Judging from the native climates of the three hosts one would expect the reverse order of hardiness than that found to be the case. Ponderosa pine has the thickest bark and grows at the lowest altitude, while limber and lodgepole pine have thin bark and grow at higher altitudes.

CHART II

CRITICAL TEMPERATURES IN RELATION TO HOST

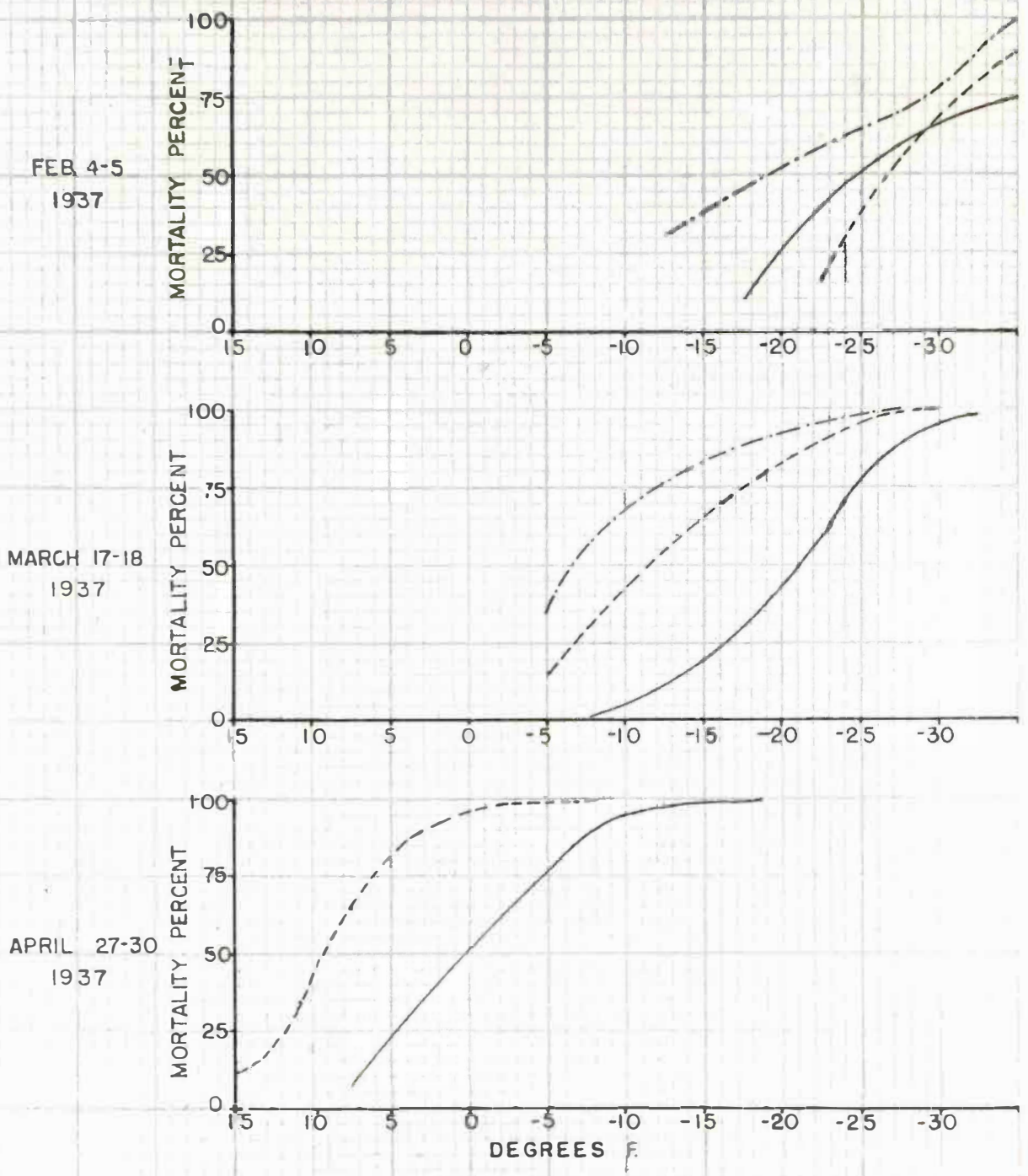


CHART II CONT.

CRITICAL TEMPERATURES IN RELATION TO HOST

JUNE 15-17
1937

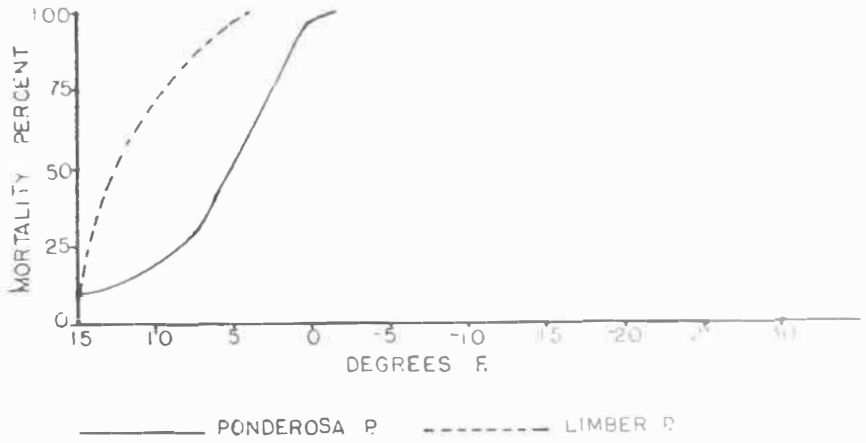
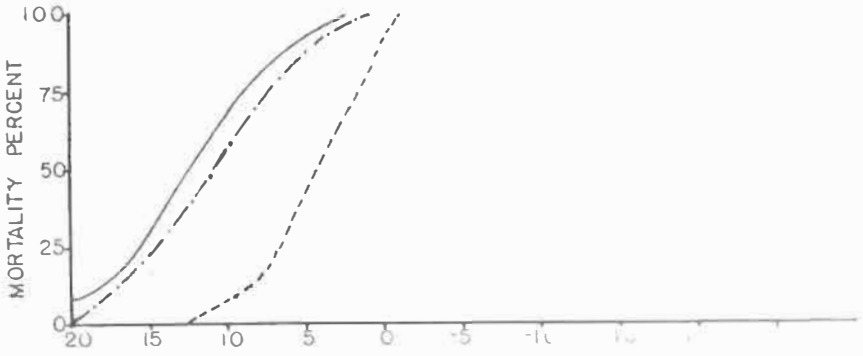


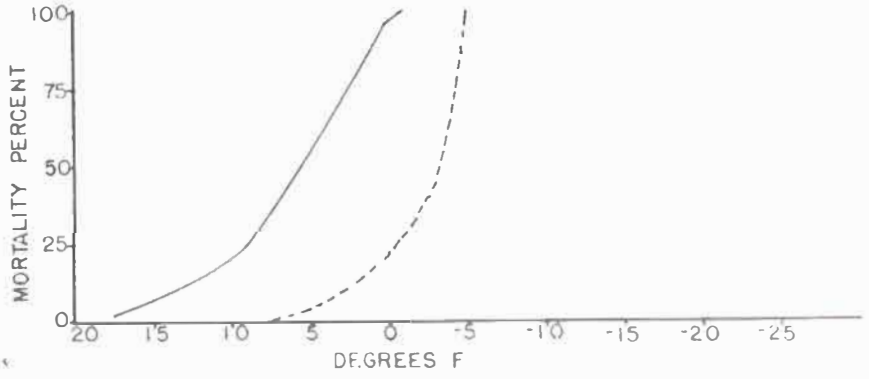
CHART III

CRITICAL TEMPERATURES IN RELATION TO STAGE OF DEVELOPMENT

GREENHOUSE
REARED
MARCH 19-24
1937



JUNE 15-17
1937



LARVAE PUPAE ADULTS

Critical Temperatures in Relation to Stage of Development

The Black Hills beetle normally overwinters in the larval stage and is therefore the only stage subject to the effects of freezing temperatures. However to make the experiment more complete, tests were made on March 19-24 on pupae and adults reared in the Entomology greenhouse of Colorado State College, by Fred Lewis; and also on June 15-17 on pupae collected in the field.

Chart III presents the relation of critical temperatures to stage of development. The pupae were 5 to 9 degrees more cold-hardy than the larvae and adults. The adults were slightly more cold-hardy than the larvae. A few of the Black Hills beetle had just started to change to the adult stage when the June 15-17 tests were made and were not sufficiently numerous to make a test of their cold-hardiness at that time.

Note also on Chart III that the insects collected in the field and tested on June 15-17 were more hardy than those reared in the greenhouse.

Summary of Weather Records

A recording thermograph and a mercury U tube type combination maximum and minimum recording thermometer were installed in the storage shelter at Allenspark on December 1. The needles in the recording thermometer sometimes followed down with the fall of the mercury columns and there was no check on the accuracy of the thermograph at the two extremes of temperature. However, the thermograph was checked with a precision thermometer several times during the winter and should have given satisfactory records.

A table in the appendix (page33) contains the daily maximum and minimum temperatures taken from the thermograph charts from December to May inclusive. The temperatures are summarized in the following table.

Summary of Temperatures at Allenspark, Colorado
Dec. 1, 1936 to May 29, 1937, incl.

Month	Maximum Temperature Deg. F.	Date	Minimum Temperature Deg. F.	Date
December	52	14	12	31
January	48	10	-13	7 & 8
February	42	24	0	8
March	51	6	- 1	27
April	62	15	13	8
May	67	27 & 28	21	3

The minimum for the period was -13° F. which occurred on January 7 and 8. The winter was described by the natives of Allenspark as a cold one with extended cold periods. The records, however, do not show

any excessively low temperatures.

The lowest recorded temperature over a period of 42 years at Longs Peak Inn, which is 6 miles north of Allenspark and 500 ft. higher then Allenspark, was -41° F. which occurred on February 7, 1933. Some years the minimum has not gone below -7° F. Unfortunately the records at Longs Peak Inn were discontinued during the past winter.

Rearing of Black Hills Beetle in Greenhouse

Fred Lewis, a senior in entomology at Colorado State College, reared D. ponderosae through several generations as part of a problem course for credit. There were some interesting observations that are worth including in this report.

He was able to rear the insects through two complete generations without interruption or a dormant period. This indicates that winter dormancy is induced by the environment, probably temperature, and is not a necessary period in the life cycle as is the case with some insects.

The Black Hills beetle is quite adaptable to artificial rearing in the greenhouse, a feature which may be useful in making later studies.

SUMMARY AND CONCLUSIONS

The first year records on the effect of low temperatures on D. ponderosae indicate that it is a very hardy insect and that low temperatures may not be a limiting factor. However the results obtained during this season should be confirmed before definite conclusions are made. Unseasonable low temperatures may be important, a point which can be cleared up after more tests have been made during the fall and spring.

The insulating values of the bark of the three hosts in this region should be determined. The tests carried out thus far have been with naked larvae. They undoubtedly can survive lower air temperatures when under the bark than when exposed in the petri dishes. With the potentiometer just recently purchased temperatures beneath the bark can be recorded with thermocouples.

The Black Hills beetle occurs over a large region and comparison of the cold-hardiness from different parts of its distribution should be made.

The effect of the "quantity" factor of cold, or extended periods of low temperatures, should also be determined. Extended cold periods should kill a higher percent than short periods of equal temperature.

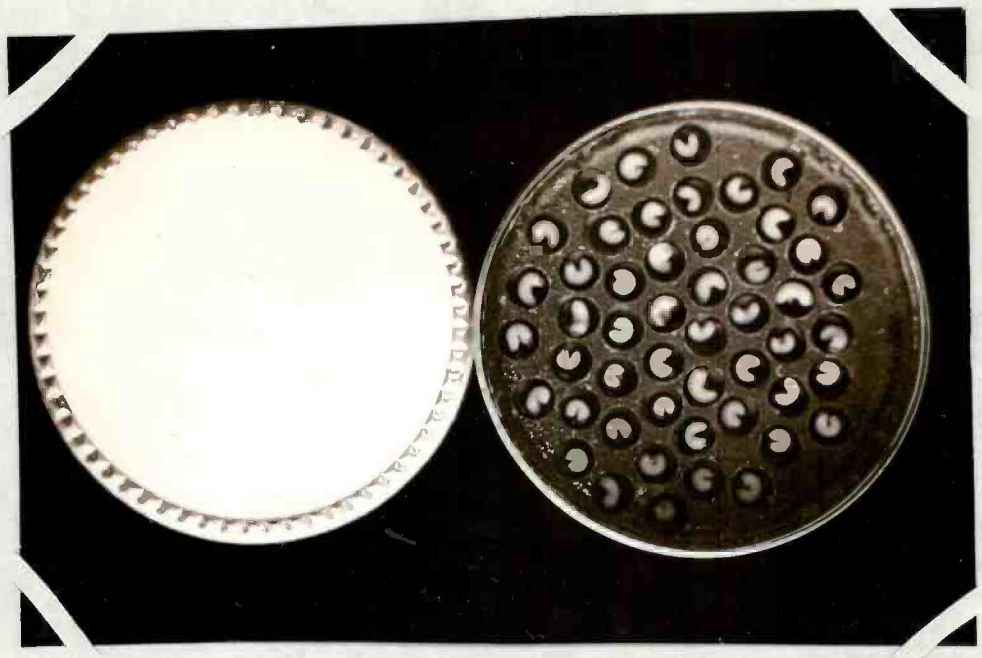


Fig. 1. Ointment tin with larvae in paraffin cells used to collect larvae for tests.

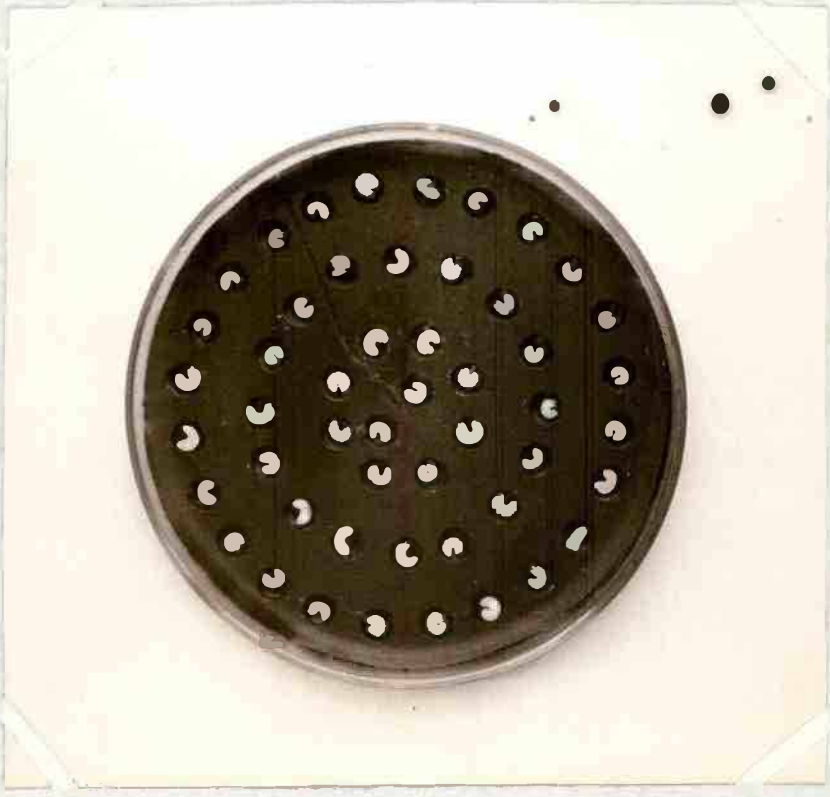


Fig. 2. Petri dish with 50 larvae in paraffin cells used to expose larvae to low temperatures.

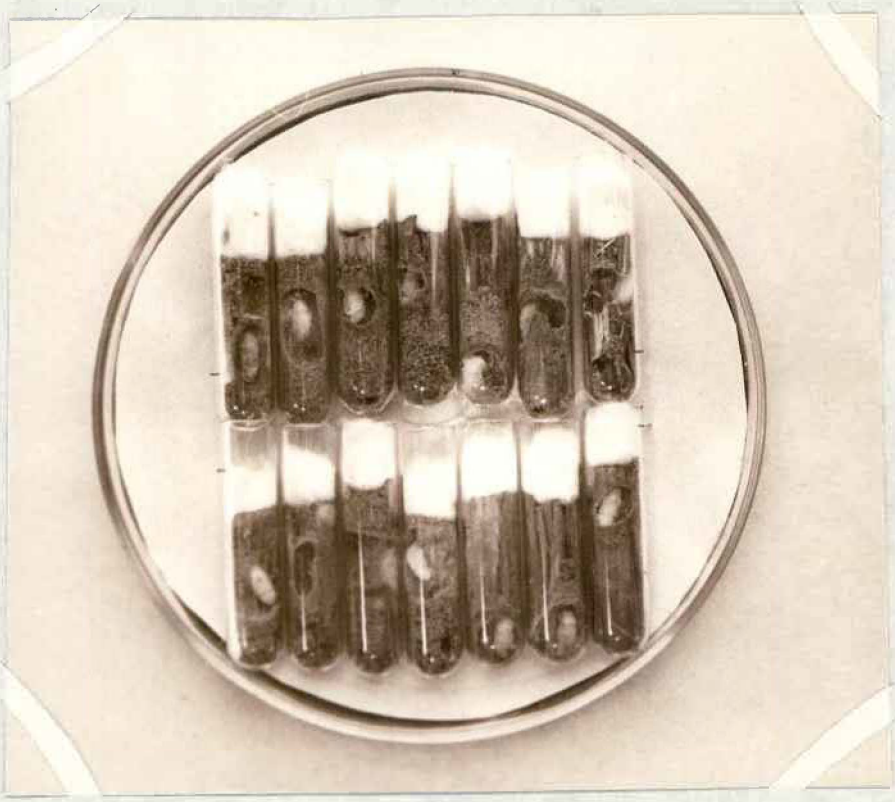


Fig. 3. 9x36 mm. vials with phloem and pupae that developed from larvae placed in vials 2 weeks earlier.

APPENDIX

Test No. 1. October 28 and 29, 1936.

Material and Procedure:

Exposure material collected October 27, 1936 from ponderosa pine at Meeker Park, Colorado. Larvae removed from bark in field and placed in petri dish cells. Petri dish containers brought to Fort Collins packed in snow, held in a refrigeretor at 35° to 40° overnight and exposure made the following day. Exposure time 8 hours and 15 minutes.

Results:

Temperature Deg. F.	No. Larvae in Sample	Mortality Percent
15	50	4
10	50	4
5	50	0
0	50	2
- 2⅓	50	22
- 5	50	30
- 7½	100	76
- 10	100	100
Check	50	0

Test No. 3. December 11-15, 1936.

Material and Procedure:

Exposure material was hauled down from Allenspark, Colo. on December 9, and the larvae removed from bark in a warm room on December 10 and placed in petri dish cells and stored in a refrigerator at 35° to 40° until used. The infested logs and the larvae after they were removed from the bark were not left in the warm room longer than necessary to make the collection. As soon as a dish was filled with 50 larvae it was placed immediately in a refrigerator. Exposure time 2 hours and 15 minutes. The sources of the infested material are as follows: (1) Ponderosa pine selected from infestation on Deer Ridge, near Allenspark, Colo., on Nov. 2 and 3, and stored in a shed at Allenspark. Elevation approximately 8,500 ft. (2) Limber pine selected from infestation on Boulder Ridge, Roosevelt National Forest, on Nov. 4 and taken to Allenspark on Nov. 14. Elevation approximately 8,500 ft. (3) Lodgepole pine selected from infestation on Mullison Creek, Medicine Bow National Forest, on Nov. 18., slabs taken from trees 12"-18" DBH., packed out and covered with hot paraffin and taken to Allenspark, Colo., for storage. Elevation approximately 8,800 ft.

Test No. 3 -- continued

Results:

Temperature Deg. F.	HOST-PONDEROSA PINE: No. Larvae in Sample	HOST-PONDEROSA PINE: Mortality Percent	HOST - LIMBER PINE: No. Larvae in Sample	HOST - LIMBER PINE: Mortality Percent	HOST-LODGEPOLE PINE: No. Larvae in Sample	HOST-LODGEPOLE PINE: Mortality Percent
-15	50	10	50	16	50	8
-17⅓	50	12	50	4	50	10
-20	50	10	50	4	50	36
-22⅔	50	42	50	16	50	34
-25	50	30	50	20	50	54
-27⅓	50	6	50	20	50	58
Check	200	1.5	150	1.3	200	4.5

Test No. 5. February 4 and 5, 1937.

Material and Procedure:

Ponderosa pine larvae collected from infestation on Mills' property, Longs Peak Inn, Colo., on Feb. 1 and larvae stored in ointment tin cells out of doors until used. Infested sections of limber and lodgepole pine stored at Allenspark were brought to Fort Collins on Feb. 1, larvae were removed in warm room on Feb. 2 and stored in petri dish cells out of doors until used. Temperature of cabinet was not low enough to start tests on Feb. 3 so larvae were held over and tests started on Feb. 4.

Duplicate samples were run on the ponderosa pine larvae and half placed in a refrigerator at 35° to 40° very soon after being taken from the low temperature cabinet. On March 4 the larvae were removed from the refrigerator and placed in office at 70° + F. Mortality counts were taken at 24-hour intervals from March 4.

Test No. 5. -- continued

Results:

Temperature Deg. F.	HOST - PONDEROSA PINE: Room Temp. Larvae 1/ No. Larvae in Sample	Mortality Percent	Refrigerator Larvae 2/ No. Larvae in Sample	Mortality Percent	HOST - LODGEPOLE PINE: No. Larvae in Sample	Mortality Percent	HOST-LODGEPOLE PINE: No. Larvae in Sample	Mortality Percent
-12½	50	16	50	6	50	12	50	32
-15	50	2	50	4	50	18	50	46
-17½	50	10	50	6	50	22	50	46
-20	50	24	50	20	50	22	50	46
-22½	50	46	50	18	50	16	50	62
-25	50	58	50	24	50	40	50	44
-27½	50	48	50	38	50	68	50	68
-30	50	70	50	28	50	48	50	72
-32½	50	68	50	34	50	88	50	94
-35	50	66	50	48	50	80	50	98
Check	150	4.7	100	5	150	21.3	50	32

1/ Larvae placed at room temperature immediately after being taken from low temperature cabinet.
2/ Larvae stored in a refrigerator, approximately 40° F., for 4 weeks after being taken from low temperature cabinet. Mortality counts taken at 24-hour intervals after being placed at room temperature following the 4 weeks in cold storage.

Test No. 6. February 18 and 19, 1937.

Material and Procedure:

The larvae were collected from ponderosa pine in the field on Mills' property near Longs Peak Inn, Colo., on Feb. 16. Larvae transported in paraffin cells in ointment tins packed in snow and transferred to petri dishes with paraffin cells on Feb. 17. The larvae were stored out of doors and not allowed to warm up.

This test was run to determine whether the larvae subjected to low temperatures are able to continue development normally. Samples of 50 larvae each were placed in 9x36 mm. vials with fresh phloem within a half day after being removed from the low temperature cabinet. The vials were stoppered with cotton and placed in large ointment tins. Only the larvae that were able to feed and develop into adults were considered as normal in making the mortality counts.

Results:

Temperature Deg. F.	Larvae in Petri Dishes: No. Larvae in Sample	Mortality Percent	Larvae in Vials with Phloem: No. Larvae in Sample	Mortality Percent
-15	50	4	50	8
-20	50	4	50	18
-25	50	16	50	66
-30	50	40	50	100
-35	50	30	50	96
Check	100	5	50	0

Test No. 7. March 17 and 18, 1937.

Procedure and Material:

Infested logs of ponderosa, limber, and lodgepole pine that had been stored at Allenspark, Colo. were brought to Fort Collins on March 15. The larvae were removed from the logs in a warm room on March 16, stored in ointment tins with paraffin cells and packed in ice until used. The insects were left in the warm room no longer than necessary to make the collection.

Duplicate samples of larvae were exposed at each temperature and half put in individual 9x36 mm. viels with phloem 2 to 18 hours after exposure to determine whether the frozen larvae were able to continue development.

Two changes were made in Test No. 7 and the following tests. The total of the larvae from each host was placed in a glass beaker and mixed before they were sorted into samples of 50. This should give more uniform samples. As soon as the "immediate" counts were made on the larvae after being exposed, the dishes were placed in containers packed in ice where they were left for 2 hours and then removed to room temperature. This procedure may have eliminated some shock caused by a rapid rise in temperature.

Test No. 7. -- continued.

Results:

Temp. Deg. F.	HOST - PONDEROSA PINE				HOST - LIMBER PINE				HOST - LODGEPOLE PINE			
	Larvae in Petri Dishes		Larvae in Vials with Phloem		Larvae in Petri Dishes		Larvae in Vials with Phloem		Larvae in Petri Dishes		Larvae in Vials with Phloem	
	No. Larvae in Sample	Mortality %	No. Larvae in Sample	Mortality %	No. Larvae in Sample	Mortality %	No. Larvae in Sample	Mortality %	No. Larvae in Sample	Mortality %	No. Larvae in Sample	Mortality %
- 5	50	2	50	4	50	10	50	16	50	28	50	34
- 7½	50	2	50	0	50	16	50	34	50	52	50	60
-10	50	2	50	10	50	36	50	42	50	64	50	74
-12½	50	2	50	14	50	50	50	58	50	78	50	76
-15	50	10	50	14	50	52	50	68	50	68	50	78
-17½	50	16	50	34	50	52	50	72	50	82	50	92
-20	50	22	50	46	50	80	50	82	50	84	50	86
-22½	50	14	50	52	50	76	50	92	50	70	50	94
-25	50	26	50	80	50	74	50	94	50	78	50	96
-27½	50	32	50	92	50	82	50	98	50	86	50	100
-30	50	18	50	96	50	80	50	100	50	92	50	100
-32½	50	42	50	98	50	74	50	100	50	90	50	100
Check	100	4	50	8	100	8	50	10	50	6	50	12

Test No. 8. March 19-24, 1937.

Material and Procedure:

The insects for this test were taken from ponderosa pine reared in the entomology greenhouse by Fred Lewis, a senior at Colorado State College. The insects were the progeny of adults that developed from infested ponderosa pine brought into the greenhouse during December 1936.

Results:

Temperature Deg. F.	LARVAE		PUPAE		CALLOW ADULTS	
	No. Larvae in Sample	Mortality Percent	No. Pupae in Sample	Mortality Percent	No. Adults in Sample	Mortality Percent
20	25	8	--	--	25	0
17½	50	14	--	--	66	13.6
15	25	32	25	0	25	16.0
12½	25	56	25	0	25	40.
10	25	76	25	8	25	68.
7½	25	52	25	16	25	52.
5	50	96	29	48.3	50	98.
2½	25	100	25	64.0	25	84.
0	75	100	71	94.4	70	100.
- 2½	--	--	25	100.	--	--
Check	50	12	29	3.4	27	0

Test No. 9. April 27-30, 1937.

Material and Procedure:

Approximately 3,000 ponderosa pine larvae were collected on Deer Ridge near Allenspark, Colo., on April 26 and 600 larvae from ponderosa pine stored at Allenspark. The larvae were placed in petri dish containers and packed in snow. Upon arrival in Fort Collins they were mixed in a glass beaker and sorted into samples of 50 each and stored in a refrigerator at 35° to 40° until used. The limber pine larvae were also obtained on Deer Ridge except for those used in tests at +10°, +12½°, and +15° which were taken from limber pine stored at Allenspark. It was necessary to make a trip to Allenspark on April 29 to obtain larvae from limber pine for the tests at 10°, 12½°, and 15°.

Test No. 9. -- continued.

Results:

Temperature Deg. F.	HOST - PONDEROSA PINE				HOST - LIMBER PINE			
	Larvae in Petri Dishes		Larvae in Vials with Phloem		Larvae in Petri Dishes		Larvae in Vials with Phloem	
	No. Larvae in Sample	Mortality Percent	No. Larvae in Sample	Mortality Percent	No. Larvae in Sample	Mortality Percent	No. Larvae in Sample	Mortality Percent
15	--	--	--	--	50	2	50	12
12½	--	--	--	--	50	20	50	22
10	50	2	50	16	50	26	50	18
7½	50	2	50	6	50	58	50	74
5	50	24	50	36	50	68	50	78
2½	50	46	50	34	50	86	50	94
0	50	54	50	46	50	96	50	92
- 2½	50	56	50	62	50	96	50	100
- 5	50	86	50	76	50	96	50	100
- 7½	50	98	50	92	50	98	50	98
-10	50	98	50	96	50	100	50	100
-12½	50	100	50	98	50	100	50	100
-15	50	100	50	100	50	100	50	100
-17½	50	100	50	98	50	100	50	100
-22½	50	100	50	100	50	100	50	100
-27½	50	100	50	100	50	100	50	100
Check	100	1	50	6	100	2	50	10

Test No. 10. June 15-17, 1937.

Material and Procedure:

Ponderosa pine larvae and pupae were collected in the field near Meeker Park, Colo., on June 14 and transported in ointment tins with paraffin cells. An infested limber pine was cut near Allenspark, Colo. on June 14 and the sections transported to Fort Collins. The larvae were collected from the sections on June 15. All larvae were stored in petri dishes in a cool basement until used.

Results:

	HOST - PONDEROSA PINE				HOST LIMBER PINE	
	Larvae		Pupae			
Temperature Deg. F.	No. Larvae in Sample	Mortality: Percent	No. Pupae in Sample	Mortality: Percent	No. Larvae in Sample	Mortality: Percent
17½	50	2	--	--	50	4
15	50	10	--	--	50	8
12½	50	18	50	0	50	60
10	50	16	50	0	50	72
7½	50	18	50	0	50	76
5	50	54	50	4	50	98
2½	50	74	50	8	50	100
0	50	98	50	22	50	100
- 2½	50	100	50	38	50	100
- 5	--	--	50	100	--	--
- 7½	--	--	50	100	--	--
Check	100	6	100	0	100	1

SUMMARY OF DAILY TEMPERATURES AT ALLENSPARK, COLORADO, DEC. 1936 - MAY 1937.

Date	December		January		February		March		April		May	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
1	32	24	14	8	34	9	40	22	41	27	44	29
2	33	21	14	8	39	18	40	26	46	25	38	29
3	32	19	19	8	24	15	34	17	31	22	34	21
4	30	21	31	18	33	17	44	12	29	15	46	24
5	26	14	41	13	38	29	49	21	36	22	56	28
6	29	16	15	- 4	41	22	51	24	33	27	54	35
7	39	28	31	-13	35	9	33	24	27	16	56	39
8	37	26	14	-13	13	0	37	16	34	13	58	34
9	32	18	23	7	16	6	43	30	44	17	52	38
10	24	16	48	18	26	7	43	31	47	30	58	38
11	40	22	41	24	40	23	45	26	45	25	52	42
12	36	17	47	22	39	24	41	20	48	24	54	34
13	41	27	28	18	35	22	20	11	53	31	58	26
14	52	34	20	14	34	22	32	5	56	42	65	47
15	44	37	33	13	35	18	38	26	62	43	63	45
16	47	30	30	17	41	23	40	27	52	31	57	35
17	32	23	17	9	27	9	36	29	30	18	64	40
18	33	23	20	9	37	25	41	24	41	22	66	39
19	48	27	25	2	26	13	29	21	50	26	62	46
20	40	27	15	- 3	16	4	35	21	46	31	57	40
21	40	27	11	- 3	23	6	35	16	55	36	55	29
22	43	32	18	- 9	32	22	--	20	55	26	58	25
23	45	33	21	- 2	37	26	--	--	--	--	63	40
24	43	33	12	5	42	20	24	10	--	--	57	41
25	44	33	19	7	20	12	17	5	--	--	48	39
26	35	25	34	13	18	6	14	1	54	--	56	37
27	43	27	29	6	28	6	20	- 1	49	26	67	35
28	44	28	34	3	28	20	27	6	39	28	67	41
29	29	19	37	21	--	--	24	10	40	25	56	42
30	18	12	23	17	--	--	36	16	44	29	--	--
31	28	12	24	9	--	--	34	26	--	--	--	--